4. Quantification by GC/negative ion chemical ionization (NICI) MS is an extremely sensitive method to analyze F_2-IsoPs. Compounds are converted to pentafluorobenzyl (PFB) ester, trimethylsilyl (TMS) ether derivatives for this analysis. The lower limits of detection of F_2-IsoPs is in the range of 3–15 fmol using a deuteriated standard with a blank of less than 5 parts per thousand. Thus, it is not necessary to assay more than 1–3 mL of a fluid such as plasma, 0.2 mL urine, or 50–1000 mg tissue.
5. Quantification of F_2-IsoPs in biological samples using the methods outlined requires a mass spectrometer with NICI capabilities. The use of NICI MS enhances the sensitivity of the assay by orders of magnitude compared to the use of electron ionization MS and electrospray ionization MS, although the use of capillary column technology will likely make electrospray ionization MS as sensitive in the future.

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8

Formation of Apolipoprotein AI-AII Heterodimers by Oxidation of High-Density Lipoprotein

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1. Introduction

Uptake of oxidized low-density lipoprotein (LDL) by scavenger receptors allows cholesterol to be accumulated in an unregulated fashion by cells in the artery wall, an event considered pivotal to the formation of atherosclerotic plaques *(1)*. High-density lipoprotein particles (HDL) are believed to protect against atherosclerosis in part by removing excess cholesterol from the artery wall *(2)*. HDL, however, is itself susceptible to oxidation by numerous in vitro methods to an extent equal to, or greater than, LDL (reviewed in **ref. *3***). In vitro oxidation of HDL by peroxidase-generated tyrosyl radical ("tyrosylated HDL"), one mechanism of phagocyte-mediated oxidation *(4)*, results in a markedly enhanced ability of HDL to promote the mobilization of cholesterol from a variety of cultured cells *(5–7)*. If occurring in vivo, this oxidative modification of HDL may actually enhance its ability to protect against atherosclerosis.

Oxidation of HDL results in the covalent crosslinking of its two major apolipoproteins, apoAI and apoAII. Isolation of the monomeric and crosslinked apolipoprotein subspecies of tyrosylated HDL allowed us to identify the component responsible for enhanced cholesterol mobilization by these particles to be apolipoprotein AI-AII heterodimers *(8)*. Separation of the individual tyrosylated HDL protein subspecies could not be accomplished using size exclusion or reverse-phase high-performance liquid chromatography (HPLC), or fast-performance liquid chromatography (FPLC). This separation was eventually achieved using preparative electrophoresis combined with elution of

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